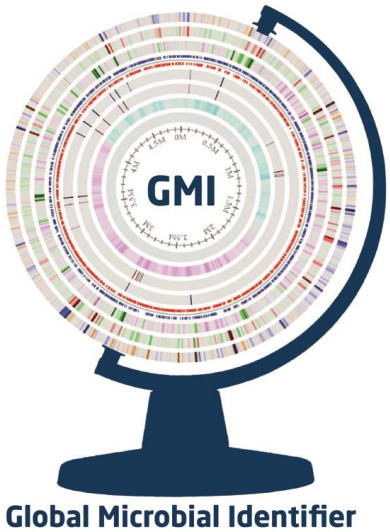
11th Global Microbial Identifier Initiative Meeting Report



16 – 18 May 2018

Geneva, Switzerland

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Acknowledgments

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Acronyms

DTU	Technical University of Denmark
FAO	Food and Agriculture Organization of the United Nations
OIE	World Organisation for Animal Health
NAFTEC	Nanyang Technological University Food Technology Centre
NGS	Next generation sequencing
PFGE	Pulsed Field Gel Electrophoresis
PT	Proficiency test
TB	tuberculosis
USFDA	US Food and Drug Administration
WGS	Whole genome sequencing
WHO	World Health Organization

Day 1: Wednesday 16th May 2018

Time	Session	Speakers
9:00	Opening	Jørgen SCHLUNDT, GMI Steering Committee Chair
	Welcome remarks	Kazuaki MIYAGISHIMA, Director, Food Safety and Zoonoses
	Overview of the day and objectives	Tim CORRIGAN, Consultant, Food Safety and Zoonoses
9:20	*Whole genome sequencing in foodborne disease surveillance: improving surveillance*	Jorge MATHEU ALVAREZ, Project Officer, Food Safety and Zoonoses
	The International Food Safety Authorities Network (INFOSAN) and recent outbreaks	Peter BEN EMBAREK, Scientist, INFOSAN Secretariat
10:00	Discussion	Moderator: Carmen Savelli, Technical Officer, INFOSAN Secretariat
10:30	Break	
11:00	*Genetic sequencing for surveillance of drug resistance in tuberculosis*	Matteo ZIGNOL, Scientist, TB Monitoring and Evaluation
	Genetic sequencing and HIV	Silvia BERTAGNOLIO, Medical Officer, HIV Treatment and Care
	Genetic sequencing and influenza	Wenqing ZHANG, Manager, High Threat Pathogens
12:00	Discussion	Moderators: Matteo ZIGNOL, Scientist, TB Monitoring and Evaluation; Carmem PESSOA DA SILVA, Medical Officer, AMR Surveillance
12:30	Lunch	
13:30	*Implications for emerging pathogens*	Sylvie BRIAND, Director, Infectious Hazard Management
	Pandemic Influenza Preparedness (PIP) Framework	Anne HUVOS, Manager, PIP Secretariat
	International legal perspectives	Steven SOLOMON, Principal Legal Officer, Governing Bodies and Public International Law
14:30	Discussion	Moderator: Sebastien COGNAT, Team Leader, Preparedness, Readiness & Core Capacity Building
15:00	Break	
15:30	*WGS across WHO*	Vaseeharan MOORTHY, Coordinator, Research, Ethics and Knowledge Management
16:00	Final discussion	Moderator: Peter BEN EMBAREK, Scientist, INFOSAN Secretariat
	Next steps and way forward	Jorge MATHEU ALVAREZ, Project Officer, Food Safety and Zoonoses
16:50	Closing	Steven MUSSER, Deputy Director for Scientific Operations, United States Food and Drug Administration
17:00	End of day	

The meeting will be followed by a reception

Day 2: Thursday 17th May 2018

Active Systems and Barriers to International Data Sharing		
Moderator: Joergen Schlundt		
08:30-08:40	Welcome – the future of WGS	
08:40-09:00	Active Systems and Overcoming NGS Barriers in the Developing World	Enrique Delgado, UNAM, MX
09:00-09:20	Genomic Data Sharing under Nagoya Protocol – Future International Initiatives	George Haringhuizen, RIVM, NL
09:20-09:40	The Vision of Sharing	Eric Stevens, US FDA, USA
09:40-10:00	Metagenomic Sewage Surveillance	Frank Moeller Aarestrup, DTU, DK
10:00-10:30	Discussion Panel: Data Sharing	
10:30-11:00 Coffee Break		
Advances in the Use of WGS in Clinical Microbiology and Functional Genomics		
11:00-11:20	Biology and Epidemiology of Shiga Toxin-Producing E. coli – NGS Investigations	Eelco Franz, RIVM, NL
11:20-11:40	Practical Issues in Implementing Next-Generation-Sequencing in Routine Diagnostic Microbiology	John WA Rossen, University of Groningen, NL
11:40-12:00	Prospective Genomic Surveillance in a Clinical Environment: Tracking Resistance and its Mobilization.	Lynn Bry, Harvard Medical School, USA
12:00-12:20	The Impact of Pathogen Genomics in U.S. Public Health	Greg Armstrong, US CDC, USA
12:20-12:40	NGS Provides Functional Insight into the Survival and Persistance of Bacterial Pathogens: The Case of Salmonella	Jie Zheng, US FDA, USA
12:40-13:50 Lunch		
NGS Proficiency Testing and New Areas		
13:50-14:10	Next Generation Sequencing Technologies for Plant Pest Diagnostics	Baldissera Giovani, EUPHRESCO, FR
14:10-14:30	NGS in the Detection of Genetic Exchange in Streptococci and Staphylococci from Food, Human and Animal Sources	Christoph Jans, ETH, Zurich, CH
14:30-14:50	UNSGM PT + GMI PT Bacs	Rene Hendriksen, DTU, DK
14:50-15:10	Establishment of Quality Control in PulseNet/GenomeTrakr	Eija Trees and Ruth Timme, US CDC and US FDA, USA
15:10-15:30	CDC experience on using WGS for patient management – implications on QA/QC	Eija Trees, US CDC, USA
15:30-16:00 Coffee Break		
AMR NGS and Accreditation of NGS Labs		
16:00-16:20	AMR Genes	David L. Trees, US CDC, USA
16:20-16:40	The NCBI Pathogen Detection Browser: Integrating Antimicrobial Resistance Genotypes and Phenotypes	Bill Klimke, NCBI, USA
16:40-17:00	Tracking the Resistome in One Health Surveillance	Patrick McDermott and Heather Tate, US FDA, USA
17:00-17:20	The EUCAST Consultations on WGS for Predicting Antimicrobial Susceptibilities	Matthew Ellington, Public Health England, UK
Conclusion of Day 2		

Day 3: Friday 18th May 2018

NGS in One Health – Surveillance and Investigation		
08:30-08:50	Application of WGS in Food Establishments: One Health Context	Ivan Nastasijevic, Institute of Meat Hygiene & Technology, CS
08:50-09:10	The Impact of NGS at the Intersection of Good Agricultural Practices and Human Food Consumption	Rebecca Bell, US FDA, USA
09:10-09:30	EFSA moving on: WGS activities for Food Safety in a European context	Beatriz Guerra, EFSA, EU
09:30-09:50	Metagenomic Approaches for Complete Char. of Human Enteric Diseases	Heather Carleton, US CDC, USA
09:50-10:10	Genomes from Metagenomes: Deciphering complex communities	Stephan Schuster, Nanyang Technological University, Singapore
10:10-10:30	WGS – the One Health linkage	Eric Brown, US FDA, USA
10:30-11:00 Coffee Break		
Use of NGS in Clinical and Public Health Virology		
11:00-11:20	Virome Profiling of Sewage for Human Disease Surveillance	My Phan, Erasmus MC, NL
11:20-11:40	One Health Surveillance and Risk Prediction in Influenza	Ron Fouchier, Erasmus MC, NL
11:40-12:00	Bringing NGS to Diagnostic Virology	Sander van Boheeman, Erasmus MC, NL
12:00-12:20	Fast and Cost-effective Sequencing of RNA Virus Genomes in Clinical Samples	Alban Ramette, University of Bern, CH
12:20-12:40	Viromes As Genetic Reservoir for the Microbial Communities in Food-Associated Environments: A Focus on Antimicrobial-Resistance Genes	Diego Mora, University of Milan, ITL
12:40-13:00	GMI Proficiency Testing- Virus	Andreas Nitsche, RKI, DE
13:00-14:10 Lunch		
14:10-16:10	WG 1-4 Break-out Session	WG 1-4 Chairs
16:10-16:40 Coffee Break		
16:40-16:50	WG1 Outcome	Joergen Schlundt, NAFTEC, SG
16:50-17:00	WG2 Outcome	Bill Klimke, NCBI, USA
17:00-17:10	WG3 Outcome	Ruth Timme, US FDA, USA
17:10-17:20	WG4 Outcome	Rene Hendriksen, DTU, DK
17:20-17:50	Concluding Discussion – incl. suggestions for GMI12 Venue	
Conclusion of Day 3		

Day 1: Wednesday 16 May 2018

WHO Initiatives using Whole Genome Sequencing as a tool for Global Health
Organized by the World Health Organization during the GMI 11 Meeting

Background

Whole Genome Sequencing (WGS) allows the identification and characterization of microorganisms with a level of precision not previously possible. As the most sensitive and specific tool available today, it is revolutionizing the way in which countries detect, assess, investigate, manage and monitor disease threats. WGS enables cross-referenceable typing systems across animal, environmental, food and human sectors. WGS is a technology with the ability to more rapidly and accurately identify the source of an outbreak and track its evolution allowing for swift responses to be mounted, preventing cases and saving lives. It is also fast becoming an important tool for surveillance of communicable diseases including antimicrobial resistance (AMR). Realizing the true power of WGS as a tool for global health is dependent on the open and widespread sharing of pathogen sequence and relevant metadata.

The World Health Organization (WHO) believes WGS technology will greatly shape the management of infectious diseases in the longer term. Many programmes across WHO are already applying this technology in projects and initiatives using these tools at regional and national levels to address public health issues.

An understanding of the full scope of WGS activities in WHO is crucial to advance discussions on WHO's role in future work in:

- supporting countries to develop the necessary infrastructure to exploit WGS;
- encouraging global timely and open sharing of sequence and metadata; and
- developing innovative approaches to equitable benefit sharing, whether monetary or non-monetary.

Objectives

Using the occasion of GMI 11, WHO will organize a one-day event to:

- inform GMI 11 participants of current WHO programmes utilizing WGS technology;
- discuss WHO's work with Member States on coordinating access to data and the benefits of data sharing; and
- identify areas of potential future work for WHO and its partners.

Day 2: Thursday 17th May 2018

Active Systems and Overcoming NGS Barriers in the Developing World
Enrique J. Delgado Suárez, Faculty of Veterinary Medicine, National Autonomous University of Mexico, Mexico

WGS has proven to be a robust tool to improve the control of infectious diseases. Despite its cost has dropped significantly, the use of NGS in developing countries is still discrete. In these nations, active epidemiological surveillance is seldom practice, even with traditional methods of microbial identification. Generally, only the most severe cases that require patients' hospitalization involve a more thorough investigation of the involved pathogen, usually in regard to its antimicrobial resistance. However, these isolates often remained in the hospital's collection and the associated information is not further used for epidemiological purposes. There are multiple factors associated with this situation. In Mexico, for instance, it would require a profound amendment of current laws and regulations. Although some governmental institutions have a growing collection of pathogen isolates, the public sharing of this information is restricted by law. Moreover, research capacity in this area is limited, as well as government policies stimulating the application of funds for these purposes. Still, there has been some important investments in government laboratories, mostly due to the pressure of the intense commercial exchange of foods. Therefore, integrating governments into the GMI initiative may be the fastest way to overcome NGS barriers in the developing world.

Main messages

- As commonly observed in developing countries, there is a lack of active epidemiological surveillance.
- Recent data show a significant proportion of foodborne diseases (92% of intestinal infections) remain unknown and classified as "other organisms".
- Some progress in the use of NGS has occurred at the governmental level, driven by intense food trade.

Main Q&A messages

- NGS for foodborne surveillance would be prioritized firstly on the top five identified foodborne diseases: (1) amoebiasis, (2) nontyphoidal salmonellosis, (3) other protozoan infections, (4) typhoidal salmonellosis and (5) shigellosis.
- For practical purposes, developing countries are likely to apply NGS for tuberculosis and HIV before common use in testing of foodborne and enteric diseases.

Genomic Data Sharing under Nagoya Protocol – Future International Initiatives
George Haringhuizen, National Institute for Public Health and the Environment, The Netherlands

GMI is committed to a world where high quality microbiological genomic information from human, animal and plant domains is freely shared among all nations. The GMI mission is to build a global platform linked to an open and interactive worldwide network of databases for standardized identification, characterization and comparison of microorganisms through the storing of whole genome sequences of microorganisms, the connected metadata, and the provision of analytical facilities and shared standards. In November 2018, at the 14th meeting of the Conference of Parties (CoP) of the CBD/Nagoya Protocol (NP), the status of NGS-data is on the agenda: do NGS-data fall by nature under the scope of NP or not? The CoP issued a scoping paper for discussion on which many research institutes, funding agencies, national institutes, and international organizations gave their opinion and expressed concern, among which WHO and FAO.

If NGS-data must be considered as equivalent to tangible genomic resources, the status of open access NGS-databases will be comparable to existing Culture Collections and BioBanks that need already be compliant to the Protocol. So, how do these Biobanks cope with the Nagoya conditions and who bears the burden? What can we learn? We looked for systematic approaches and identified 4 models (American, Asian, Japanese, European) depending on who is responsible for the access and benefit sharing negotiations with the country of origin. All investigated models however end up being burdensome and compromise somehow the open and timely sharing of genetic resources, especially the fast sharing in situations of public health emergencies. Will it be possible under the NP to create exemptions or otherwise mechanisms for free, fair and fast sharing of essential information, and thus uphold the original vision and mission of GMI?

Main messages

- The Nagoya Protocol took effect in 2014, under the Convention on Biological Diversity.
- The Nagoya Protocol regulates the use of genetic resources whereby the receiving party holds the responsibility for acquiring legal consent from the originating country's government i.e. prior informed consent and mutually agreed terms.
- The status of NGS-data falling under the Nagoya Protocol remains to be confirmed.
- When considering NGS-data as describing nature, data does not appear to fall under the Nagoya Protocol and the protocol may become void.
- When considering NGS-data as a de facto functional comparable to biobanks, data does appear to fall under the Nagoya protocol and four current models have been identified: (1) the non-involvement model (2) the trusted intermediary model (3) the burden sharing model i.e. ACM-NIEMA model (4) the active mediator model i.e. Japanese culture collection.
- Current existing models do not serve the objectives of GMI.

The Vision of Sharing
Eric Stevens, US Food & Drug Administration, United States of America

The importance of sharing WGS data cannot be overstated. Making the data available to all sectors within the One Health framework in real time enhances the impact of WGS for food safety and public health. This presentation will demonstrate the power of sharing WGS data and relate its impact on our understanding of human genomic variation and molecular evolution to what it can do for microbial populations. Examples of how WGS has been used and could be used will be discussed as we work towards the vision of making WGS data sharing a global reality.

Main messages

- The success of the human genome project comes from the ability to share human genome data and prepare large reference populations of the sequenced data.
- The project enabled researchers to better understand human genetic variation in relation to human health and disease, improving disease diagnosis and ultimately saving lives.
- As exhibited by the human genome project, building reference populations of sequenced microbial data would enable better understanding of microbial diversity as well as provide the opportunity to compare human genomic data with microbial data e.g. to better understand listeriosis as well become better informed about agricultural practices.

Global surveillance of antimicrobial resistance
Frank Moeller Aarestrup, Technical University of Denmark, Denmark

Antimicrobial resistance (AMR) is one of the most serious global public health threats, however, obtaining representative data on AMR for healthy human and animal populations are difficult. We have developed online bioinformatics tools and abilities for analyzing and sharing whole genome sequencing data in a standardized way facilitating open and global sharing of data. There can be several barriers for sharing data from clinical infections and isolates from clinical infections provides limited information on healthy human and animal populations. We have developed metagenomics approaches allowing determination of the abundance and diversity of all AMR genes in metagenomics samples and are now using this to study the occurrence and transmission of AMR globally. Sampling is currently ongoing in +100 countries and initial results from a sub-set of the data will be presented. Part of the raw sequencing data are shared in real-time for everybody to use.

Main messages

- Present collection of sewage samples provides an alternative way to conduct large scale global hot spot surveillance pertaining to prevalence of antimicrobial resistance in the healthy population with limited ethical concerns i.e. no preapprovals required.
- Analysis of findings show antimicrobial resistance is significantly associated with antimicrobial use, extremely strongly associated with the human development index (measurement used by WHO) specifically indicators of sanitation and local health systems.
- Sewage samples can be a useful means to measure other information including the prevalence of viruses, parasites, illegal drugs and population information that minimizes human bias that comes with questionnaires.
- Global predictions can be made through combining sewage surveillance data with data from The World Bank.

Main Q&A messages

- The context of which WGS data was obtained should always be taken into consideration to avoid assigning incorrect blame and punitive actions
- Ignorance and lack of compliance in re to the Nagoya Protocol can result in heavy fining. It is recommended to make use of the possibilities of the Nagoya Protocol.

Biology and Epidemiology of Shiga Toxin-Producing *E. coli* – NGS Investigations
Eelco Franz, National Institute for Public Health and the Environment, The Netherlands

Shiga toxin-producing *Escherichia coli* (STEC) are globally dispersed pathogens associated with a broad spectrum of clinical manifestations in infected humans, including diarrhoea, haemorrhagic colitis and (occasionally fatal) haemolytic uremic syndrome (HUS). STEC are generally considered zoonotic with ruminants, and particular cattle and sheep, as the main reservoirs. *E. coli* O157:H7 is the most commonly reported STEC serotype. Although evolutionary models have been developed the geographical spread of the pathogen and the extent of inter- and intra-continental transmission are still to be analysed comprehensively and quantitatively. We provide the first comprehensive global phylo-geographical analysis of STEC O157, reconstructing the phylogenetic history and global spread of the contemporary clones. Next to STEC O157 some STEC are truly emerging pathogens, including stx2fproducing STEC which represents a significant fraction of human isolates and so far only is isolated from pigeons. We conducted an in-depth genomic comparison of stx2f-carrying E. coli from pigeons and humans in order to contribute to the understanding of the ecology and epidemiology of this emerging group of STEC. We hereby identified a new hybrid *E. coli* pathotype.

Main messages

- WGS can be used to reconstruct the spread of zoonotic pathogens and better estimations could be made with more WGS data that are compiled into a global database.
- The movement of live farm animals may be a plausible explanation for the global spread of STEC O157.
- STEC is a dynamic group of pathogens with emergence of new types all the time and the use of WGS can help to predict to future movements and spread.

Practical Issues in Implementing Next-Generation- Sequencing in Routine Diagnostic Microbiology
John WA Rossen, University of Groningen, The Netherlands

Next generation sequencing (NGS) is increasingly being used in clinical microbiology. Like every new technology adopted in microbiology, the integration of NGS into clinical and routine workflows must be carefully managed. As the microbiology laboratories have to adhere to various national and international regulations and criteria for their accreditation, quality control issues for using WGS in microbiology, including the importance of proficiency testing will be discussed. Furthermore, the current and potential future place of this technology in the diagnostic hierarchy of microbiology will be presented as well as the necessity of maintaining backwards compatibility with already established methods. Finally, the question of whether WGS can entirely replace routine microbiology in the future and the tension between the fact that most sequencers are designed to process multiple samples in parallel whereas for optimal diagnosis a one-by-one processing of the samples is preferred will be addressed.

Main messages

- WGS provides detailed characterization of all kinds of samples but there is a need to find a balance between cost, quality, speed and complexity of the wet and dry lab processes.
- Implementing NGS in clinical laboratories as well as in central reference laboratories will help to reduce turnaround time and manpower in hospital-based microbiology laboratory.
- Clinical microbiology laboratories should invest in WGS to be able to implement future applications of next generation sequencing.

Prospective Genomic Surveillance in a Clinical Environment: Tracking Resistance and its Mobilization
Lynn Bry, Harvard Medical School, United States of America

Diverse vectors mobilize drug resistance in pathogens, including via conjugative plasmids and transposons. While some forms of resistance are strongly associated with specific vectors, such as transmission of Klebsiella pneumonia carbapenemases (KPC) by the Tn4401 transposon, others, such as the New Delhi metallo-beta-lactamases (NDMs), have a more diverse vectors that mediate spread. In addition to specifying capacity for mobilization of resistance within and among species, the complement of mobile vectors also provides a robust set of information to assist with outbreak and strain cluster analyses. However, the complex and repetitive nature of these vectors introduces complexities in genomic analyses, and often requires specific technical, experimental and computational approaches to resolve carrying vectors and those involving nested structures. We present clinical cases that used mobile vector information to support gene-, vector-, strain- and cluster-level analyses to provide actionable data to support infection control and clinical microbiology operations.

Main messages

- Every outbreak investigated internally has almost always used publicly available data from GenomeTrackr and other centers.
- Two example case clusters using mobile vector analyses were shared, (1) XDR *Shigella sonnei*, which was found to be associated with a broader outbreak and (2) XDR and PDR *E.coli* whereby *Enterobacter* species was found to be a common intermediate vector and transferred by conjugation with occasional methods by inter-plasmid recombination.

The Impact of Pathogen Genomics in U.S. Public Health
Gregory Armstrong, Centers for Disease Control and Prevention, United States of America

Since 2014, the US CDC, together with federal, state and local partners, has been adapting next-generation sequencing technologies in dozens of infectious-disease public health domains, such as food safety, tuberculosis control, influenza monitoring, and monitoring of antimicrobial resistance in viral, bacterial, and eukaryotic pathogens. This transition has been facilitated by the Advanced Molecular Detection (AMD) program, a $30M-per-year initiative to bring genomic sequencing, bioinformatics and related technologies bear against public health threats. The program is now in its fifth year and at a point where state and local health departments in the US are rapidly acquiring the technology and implementing it, in most cases, first for bacterial foodborne pathogen characterization, but increasingly in other areas. This talk will focus on the AMD program, how it has been fostering the adoption of pathogen genomics into the US public health system, and where the program is currently having the greatest impact.

Main messages

- AMD has funded several applications adopting WGS for the improvement of public health including work on (1) hepatitis C (2) malaria (3) legionella and (4) influenza.
- For (1), there is sufficient sequencing data to be used for surveillance and work is currently conducted to identify outbreaks, which suggest potential intervention in the community.
- For (2), amplicon sequencing is currently used to look for resistance.
- For (3), fine subtyping has provided useful data in investigating outbreaks and understanding the ecology in water systems prevalent with the pathogen.
- For (4), WGS has enabled better quality data for improved vaccine selection.
- Future directions include increased collaboration with academia for integrating sequencing data with epidemiological data.

NGS Provides Functional Insight into the Survival and Persistence of Bacterial Pathogens: The Case of *Salmonella*

Jie Zheng, Yu Wang, Elizabeth Reed, and Eric W Brown US Food &Drug Administration, United States of America

Genetic adaptations to food and the food production environment observed in *Salmonella* and other pathogens associated with foodborne illnesses have become a public health concern. This highlights the probability of evolutionary changes in these pathogens, in which a selective advantage was conferred for survival, persistence and even growth within food matrices and in the environment, increasing their propensity for morbidity and mortality. For example, sprouts contamination is one of the recurrent problems both in the United States and around the world. Between 2000 and 2016, at least 17 salmonellosis outbreaks linked to the consumption of raw sprouts were documented internationally, nine of them in the US. Most involved alfalfa sprouts, but cress, mung bean, and clover sprouts were also implicated. A metatranscriptomics approach was applied to examine *Salmonella* functions in sprout spent irrigation water (SIW). Interestingly, genes with different functions in *Salmonella* were observed to be highly transcribed at different time points during the sprouting process. This study sheds light on the active interaction of *Salmonella* with the sprout microbial community. The application of NGS in food safety will give rise to a deeper understanding of *Salmonella* adaptations to certain environmental conditions and help identify preventive control measures to inhibit pathogen growth.

Main messages

- The role of functional genomics in food safety is moving from a reactive to proactive state.
- The use of NGS to provide preventative control measures against foodborne outbreaks was exemplified by cases of *Salmonella* in tomatoes and sprouts. In the former, *S*. Newport was implicated in 6 multistate tomato outbreaks in the US between 1973-2000, 69% of which were from red, round tomatoes characterized by higher pH values than roma and grape tomatoes.
- Findings show tomato "Newport "strains came from *S*. Newport clade III whereby *S.* Newport had increased carbon catabolism and stress response compared to other serovars, and lineage III was characterized by increased virulence and restriction modification.
- The application of NGS can give rise to a deeper understanding of pathogen adaptations to certain environmental conditions.

Main Q&A messages

- Phase 2 of the AMD program includes the policy that all data must be released within a specified period of time.
- Due to limited resources e.g. manpower and slow turn around due to manual aspects of curation, NGS is currently not used clinically for individual patient care.
- Instrument manufacturers are aware of cost barriers faced especially by developing countries in accessing new technology and over the years have strived to drive down capital investment and costs of sequencing data. It is highlighted that further improvement can be made in reducing associated costs with data analysis.

Next Generation Sequencing Technologies for Plant Pest Diagnostics
Baldissera Giovani and Françoise Petter European and Mediterranean Plant Protection Organization, France

Reliable and rapid diagnostic processes are essential to support inspection activities conducted by National Plant Protection Organisations (NPPOs) in the framework of their official mandate, and to evaluate the efficacy of measures taken. Official controls aim to prevent or reduce the risk of introducing new pests through the agri-food trade and to protect consumer interests (Giovani et al., 2018). During the last decades the incidence of plant diseases has increased exponentially in terms of both numbers and severity, as a result of increased trade of plants and plant products, advances in transport technologies and the development of a complex network of global commerce (Santini et al., 2017). The European and Mediterranean Plant Protection Organization recommend more than 300 pests (any species, strain or biotype plant, animal or pathogenic agent injurious to plants or plant products) for regulation. Regulated pests are officially controlled and countries organise surveillance pre-border, at border and at places of productions on commodities that are the hosts of a given pest. By allowing the sequencing of the whole genome of (micro)organisms present on a commodity without a priori knowledge, NGS technologies can greatly support the work of official diagnostic laboratories and empower inspectors in situ. The presentation will provide an overview of the plant health sector and of the various international initiatives organised to promote and adopt this new diagnostic paradigm while taking into account specific challenges such as the detection of unknown organisms and the international sharing of data.

Main messages

- A lack of data to assess risks on plant and plant based products may lead to denial of trade.
- High throughout sequencing for plant health is not only useful for identification but also for surveillance and certification, post entry quarantine and monitoring imported commodities for new potential risks.
- Workshops and projects are currently being conducted in preparation for greater inclusion of NGS technology in plant pest diagnostics.

NGS in the Detection of Genetic Exchange in Streptococci and Staphylococci from Food, Human and Animal Sources

Christoph Jans ETH Zurich, Department of Health Sciences and Technology, Laboratory of Food Biotechnology, Switzerland

Streptococci and staphylococci are among the most important genera in relation to public and animal health. Furthermore, certain species of these genera share a strong association with food, either in the role of foodborne pathogens, but also as organisms to produce fermented food. The ability to proliferate in these various niches suggest genetic adaptation as a contributor to handle the different environments. Food-derived streptococci (Streptococcus bovis group) and staphylococci (Staphylococcus aureus) obtained in subSaharan Africa represent exotic examples to study genetic adaptation and gene exchange in comparison to a global pool of strains of animal, human and food sources. Among the *S*. bovis group, a recombinant rather than clonal history also has important implications on the reliability of classical phenotypic and single gene genotypic identification approaches. This is exemplified by genetic exchange and genome decay in the *S.* bovis group that affect basic carbohydrate metabolism such as that of lactose but also virulence features such as adhesion abilities or capsule properties. Next generation sequencing (NGS) provides the tool to investigate these adaptations in combination with gaining insights into evolution, population structure and genome plasticity presented on the examples of *S*. bovis and *S.* aureus.

Main messages

- Milk serves as a habitat for human and livestock associated *S. aureus* in Africa.
- There is a clear habitat and East-West Africa differentiation for dairy isolates in regards o S. bovis/S.equinus complex (SBSEC)
- NGS limitations: in silico predictions need biological support from the lab.
- Milk production in subsaharan Africa appears to be informal and usually takes up to 24h (in ambient temperature) before reaching the consumer. Due to religious reasons, the milk remains unpasteurized.

Proficiency Tests in genomics – for UNSGM and GMI

Rene S. Hendriksen Research Group of Genomic Epidemiology, DTU-Food. WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens and Genomics (WHO CC). European Union Reference Laboratory for Antimicrobial Resistance (EURL-AMR). Technical University of Denmark, National Food Institute, Kgs. Lyngby, Denmark

Proficiency testing (PT) is an important tool to assess data leading to ensure reliability. The Technical University of Denmark, the National Food Institute (DTU Food) has a long track record for providing PTs to WHO and EU. Recently, DTU Food has launch with partners the UNSGM PT for the Secretary-General's Mechanism for Investigation of Alleged Use of Chemical and Biological Weapons as well as maintaining the GMI PT. The purpose of the UNSGM PT is to assess laboratories ability to correctly detect and characterize a biological threat and associated genetic markers such as virulence factors and antimicrobial resistance using modern high-throughput genomic technologies, whole genome sequencing (WGS) to increase the global and national preparedness. Similarly, the GMI PT aim at facilitating the production of reliable laboratory results of consistently good quality within the area of WGS. A total of 56 laboratories participated in the UNSGM PT with 90.1% of all laboratories correctly identified the genomes to species level. Thirteen laboratories obtain an overall score above 80%. In the GMI PT, 66 laboratories participated with the majority performing well. A few laboratories were identified as being outliers due to either contamination of the reference material or poor sequencing

ability. Overall in both PTs, the programmed successfully identified the laboratories show a high level of proficiency despite the different scopes.

Main messages

- The UNSGM PT consisted of two components (1) data set of 36 single genomes and (2) metagenomics data set of a food sample conducted by a specific roster of experts and laboratories nominated by Member States.
- For UNSGM PT (1), failure to correctly identify unmodified *Shigella dysenteriae* was significant
- For UNSGM PT (1), 13 laboratories ranked an overall score of above 80% including Slovenia, China, Sweden, Germany, Portugal, UK, Norway, Switzerland, France, Singapore and USA.
- Data from the GMI PT will be summarized and published as a scientific paper as well as a GMI report.
- No GMI PT will go ahead in 2018.

Establishment of Quality Control in PulseNet/GenomeTrakr

Eija Trees[1] and Ruth Timme[2]
[1]Centers for Disease Control and Prevention, U.S.A., [2]Food and Drug Administration, U.S.A.

As whole genome sequencing (WGS) is established as the primary subtyping tool for foodborne pathogens it is imperative to implement a rigorous quality assurance / quality control (QA/QC) program to ensure the integrity and comparability of the data deposited to public or limited access repositories. PulseNet and GenomeTrakr, the two US networks sequencing clinical, food and environmental isolates for foodborne disease surveillance, outbreak investigations and attribution, are working together towards harmonized QA/QC program. The QA/QC program being established is based on a quality manual, a collection of electronic standard operating procedures for both wet and dry lab procedures that include multiple quality control points for each procedure. Minimum quality thresholds at each quality control point must be met in order for the procedure to move to the next step. New staff undergoes training that has been harmonized between the networks and for PulseNet are also required to pass a competency assessment (certification) before submitting data to the network databases. A laboratory maintains its certification status by participating in an annual proficiency test that is harmonized across both networks.

Main messages

- The terms QA/QC should not be used interchangeably as the former is focused on the process e.g. staff training, staff competency, whereby it particularly important when comparing data from a network of labs. QC is concerned with the product e.g. DNA extract, DNA library, raw reads and the implement checkpoints along the process.

CDC experience on using WGS for patient management – implications on QA/QC
Eija Trees, Centers for Disease Control and Prevention, United States of America

The implementation of whole genome sequencing (WGS) as a primary subtyping tool in public health laboratories will introduce an important paradigm shift also for the reference and diagnostic laboratories. Traditional workflows that have been used up until now to identify the species, serotype, virulence and antimicrobial resistance profiles and the strain subtype are now being replaced by one workflow based on genomic sequencing. In the United States, laboratory tests that generate data to be used to make decisions on patient management are regulated by the federal Clinical Laboratory Improvement Amendments (CLIA) established in 1988. CLIA requires laboratories to be certified by their state before they can accept samples of human origin for diagnostic testing. Before a certification can be obtained, documentation needs to be in place on assay validation, staff training, equipment maintenance, and quality control measures for assay performance. Any modifications to the workflow require full re-validation or re-verification depending on workflow change significance. Participation in two proficiency tests annually is required and certified labs are inspected every other year. At the US CDC, Enteric Diseases Laboratory Branch (EDLB) is the first, and so far the only, program to obtain CLIA certification for WGS-based diagnostics.

Main messages

- Compliance with regulatory requirements and quality standards can be challenging for NGS based assays because traditional definitions for performance characteristics do not readily translate to DNA sequence methods and data.
- CLIA states one positive control for one of the target species through the entire process which increases per sample cost.

Main Q&A messages

- The College for American Pathologists (CAP) has a lab accreditation program for NGS including general plan principles for the wet lab and bioinformatics component that may be followed as a CLIA approved framework for direct patient care. CAP may also be a useful party involved in any future dialogue re difficulties in compliance with CLIA.
- Use of NGS in plant pest diagnostics has been discussed with regulators and scientists but not yet with producers, traders and consumers. Stakeholders may be more willing to participate if it provides potential advantages to their business goals.

The NCBI Pathogen Detection Browser: Integrating Antimicrobial Resistance Genotypes and Phenotypes
William Klimke, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, United States of America

The NCBI Pathogen Detection pipeline takes raw sequencing data from a surveillance network of public health labs sequencing food and clinical pathogen isolates and generates publicly available reports. The pipeline takes the raw sequencing data, assembles, annotates, and clusters the isolates into phylogenetic trees to aid outbreak and traceback investigations. The annotated assemblies are checked for the presence of antimicrobial resistance genes/proteins using a process built by NCBI called AMRFinder. AMRFinder uses a set of curated proteins and hidden markov models (HMMs) to assign functional names to proteins. AMRFinder has recently been tested on 6,242 isolates from the National Antibiotic Resistance Monitoring System (NARMS). These isolates were a mix of different species for which antibiotic susceptibility test (AST) were done. Comparison of genotype predictions from AMRFinder and the 89,318 susceptibility tests performed across the set of isolates showed that

AMRFinder made a call consistent with the phenotype 98.1% of the time. The genotype calls and phenotype information are added to the Pathogen Isolates Browser: https://www.ncbi.nlm.nih.gov/pathogens/isolates#/search/

Main messages

- Due to the scale of data coming into the system, some parts of the NCBI pathogen pipeline are slowing down. NIH has recently developed software to speed up the clustering and tree construction, which will be implemented later this year.
- Future directions include plans to add point mutations, and all pathogenic bacteria (*S. aureus* being notably mentioned) and mobile elements.

Tracking the Resistome in One Health Surveillance
Patrick McDermott* and Heather Tate, US Food & Drug Administration, United States of America

In the US, the National Antimicrobial Resistance Monitoring System (NARMS) has been using WGS of Salmonella as a tool of routine surveillance since 2013. To date, NARMS has generated MIC and WGS data on over 10,000 Salmonella isolates from food animals, 9,000 from human clinical cases, 6,000 from retail meats, and 1,400 from imported foods. Analysis of this dataset showed that the presence of known resistance determinants is very highly correlated with clinical resistance, indicating that WGS data can be used to predict resistance in Salmonella strains lacking traditional antimicrobial susceptibility data. To make these data accessible, the NARMS program has launched Resistome Tracker and other online tools that provide visually informative displays of antibiotic resistance genes. Resistome Tracker harvests resistance gene information on a weekly basis from genomic data deposited at NCBI. It presents the resistome data using interactive dashboards that allows users to customize visualizations by antibiotic drug class, compare resistance genes across different sources, identify new resistance genes, and map selected resistance genes to geographic region. The tool also provides alerts about new resistance traits as they emerge in a region or source to provide early warning on emergent trends.

Main messages

- NARMS is primarily concerned with trending changes in genome and resistomes in the food supply.
- Nucleotide surveillance is a single assay that provides the highest practical resolution of structural traits in microbial members of an ecosystem.
- The addition of WGS to phenotypic analysis has been transformative for surveillance e.g. enabling better understanding of antimicrobial resistant/antimicrobial usage relationship and tracking changes from AMU policy bans.

The EUCAST Consultations on WGS for Predicting Antimicrobial Susceptibilities

Matthew J Ellington, Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit, National Infection Service, Public Health England, London, United Kingdom

WGS offers the potential to predict antimicrobial susceptibility from a single assay. The European Committee on Antimicrobial Susceptibility Testing established a subcommittee to review the current development status of WGS for bacterial antimicrobial susceptibility testing (AST). Barriers and issues to the wider adoption of the technology were considered, including whether epidemiological cut-off values (ECOFFs) or clinical breakpoints are the most appropriate comparators for genotypic and phenotypic data. Analysis showed that the use of WGS-inferred AST for guiding clinical decision was not supported by the evidence base, but this area should be a funding priority if it is to become a rival to phenotypic AST.

Main messages

- Findings of the 2015 published review note a growing number of specific antibiotic and bacteria studies exhibiting high concordance between WGS and phenotypic based AST results e.g. above 90% for *S. aureus*, while for others it was harder to predict e.g. 88% concordance for *P. aeruginosa*
- Systematic sources of error affecting phenotypic/WGS correlation include major errors in WGS prediction when there is incomplete understanding of genotypic basis of phenotypic resistance

Main Q&A messages

- According to EFSA there are discussions to include molecular subtyping in new 2020 legislation for AMR monitoring, meanwhile MIC testing remains mandatory in current practices.

Day 3: Friday 18th May 2018

Application of WGS in Food Establishments: One Health Context

Ivan Nastasijevic*, Brankica Lakicevic and Branko Velebit Institute of Meat Hygiene and Technology, Serbia

Food safety is a global concern, and consumers have the right to safe and nutritious food. However, the estimated burden of foodborne diseases at global level, e.g. 600 million food borne illnesses and 420,000 deaths from 31 major food safety hazards in 2010 (WHO, 2015), as well as at EU level, with around 356,000 confirmed human illnesses and 452 deaths (EFSA, 2017) from 5 major food safety hazards (Campylobacteriosis, Salmonellosis, Yersiniosis, STEC infections, Listeriosis) and the related social and economic costs (hospitalization, loss of income, employment and market access) remain unacceptably high. Tracking the foodborne pathogens in the food processing – distribution – retail – consumer continuum is of utmost importance for facilitation of outbreak investigation and rapid action in controlling/preventing foodborne outbreaks. Whole Genome Sequencing (WGS) has recently emerged as a new tool and offers great potential in the way we investigate, assess and manage microbiological food safety issues, food borne illnesses, including associated antimicrobial resistance (FAO, 2016). The use of WGS can facilitate the understanding of contamination/colonization routes of foodborne pathogens within the food production environment and can also enable efficient tracking of entering routes of pathogens and their distribution from farm – to – consumer. WGS is a powerful tool for obtaining genomic data, which gives a higher level of resolution discrimination, i.e. better information about genetic similarity between isolates than conventional molecular typing such as Fluorescent Amplified Fragment Length Polymorphism (fAFLP) or Pulsed Field Gel Electrophoresis (PFGE); such molecular methods only determine if isolates are the same or different but not how closely related they are genetically. Implementation of WGS is likely to be beneficial for many countries in the foreseeable future, in support of food safety management systems and foodborne outbreak investigations. In recent years, development and availability of user-friendly portable real-time devices for DNA and RNA sequencing enabling ultra-long read lengths are possible (hundreds of kb). The cost of WGS has also lowered significantly allowing its use in more routine applications since the price for bacterial genome sequencing fall to less than $50/isolate, in case that a considerable number of isolates is sequenced at the same time on a given instrument to achieve maximum economy of scale. For instance, it may encompass in-house sequencing when sequencing equipment is used for multiple applications (WGS of pathogens, starter cultures and spoilage organisms). Further, WGS is currently becoming the method of choice for characterizing isolates of the major food borne pathogens (Salmonella, Campylobacter, Listeria monocytogenes, STEC) in national reference laboratories across USA and EU. Inter-sectoral cooperation in `One Health` context between environmental, veterinary and health authorities through establishment of national public database of isolates (non-food, food and human origin) will allow the real-time exchange of information and comparison of genetic similarity between isolates of foodborne pathogens. Such approach will significantly facilitate understanding of distribution of pathogens and antimicrobial resistance along the food chain continuum and improve the effectiveness of outbreak investigations.

Main messages

- 60% of human pathogens are of zoonotic origin.
- WGS should be used at the food safety management level in terms of predicting what is important or what will be important in the near future
- The Earth Biogenome Project is a 10 year 4.7-billion-dollar project aimed to sequence the DNA of all known eukaryotic species on Earth.

The Impact of NGS at the Intersection of Good Agricultural Practices and Human Food Consumption
Rebecca Bell, US Food & Drug Administration, United States of America

An estimated 14% of all foodborne outbreaks reported in North America are attributed to the consumption of raw or minimally-processed fruits and vegetables. In particular, consumption of fresh tomatoes has been linked to numerous foodborne outbreaks involving various serovars of *Salmonella enterica*. The association of Salmonella with tomatoes is ecologically complex and not yet fully understood. Successful mitigation strategies will depend on extensive environmental monitoring and understanding of the unique adaptive changes acquired by these human pathogens in the plant environment. An extensive environmental monitoring program for Salmonella has been ongoing along the VES since 2009. Next generation sequencing (NGS) has aided in the understanding of the ecology and biology within this non-host environment in several ways from traceback during outbreaks to functional genomics that may lead to new mitigation strategies to reduce or prevent contamination of fresh produce.

Main messages

- Besides source tracking and traceback, WGS is a useful tool in providing ecological insights, root cause insights and fitness insights on microorganisms.

EFSA Moving On: WGS activities for Food Safety in a European context
Beatriz Guerra, Valentina Rizzi, Ernesto Liebana European Food Safety Authority, EFSA, Parma, Italy

Given the growing importance of WGS analysis for food safety (multinational foodborne outbreak investigations, surveillance/monitoring fields including AMR, use for approval of regulatory products including GMOs), the European Food Safety Authority (EFSA) has recognized the need to move on. In EFSA, several WGS activities, grouped under a WGS Umbrella project, are currently taking place. These include, among others, i) “In house” capacity building; ii) EC Mandates: ECDC-EFSA are exploring the possibility to extend the current collection and analysis of molecular typing data from foodborne pathogens in the joint ECDC-EFSA molecular typing database to WGS data; and EFSA is exploring the possibility to use molecular typing methods including WGS for the harmonized monitoring of AMR in bacteria transmitted through food. Iii) Funding research projects (ENGAGE, INNUENDO, GENCAMP, Liseq, others). iv) Using WGS analyses (performed by the EURLs) to support EFSA routine activities: e.g. for the European Summary Reports on AMR data (with EURL-AR and MSs to support the data quality and detection of emerging resistance mechanisms/resistant clones), as well as for rapid outbreak assessments (with ECDC and EURLs). v) Collecting information on the availability of WGS for the main foodborne pathogens in animals, food, feed and their related environment in European food safety and veterinary laboratories.

Main messages

- Published survey in 2016 show WGS activities for food/waterborne pathogens were carried out in 44% of EU national reference laboratories. Since then, the use has grown.

Metagenomic Approaches for Complete Characterization of Human Enteric Diseases
Heather Carleton US CDC, United States of America

PulseNet, the U.S. national molecular foodborne surveillance program, is preparing for a future without isolate culture. Current molecular surveillance relies on isolates which are less frequently submitted to public health laboratories due to increased use of culture independent diagnostic methods (CIDTs) by the clinical laboratories. PulseNet is currently developing pathogen detection, subtyping and strain characterization methods that by themselves are independent of cultures. PulseNet is pursuing two approaches: (1) near term - amplicon sequencing targeting specific pathogens including enough markers to provide sufficient resolution for outbreak detection and investigation similar to the cgMLST approach to subtype cultures of specific pathogens; and (2) long term- non-specific metagenomics by shotgun sequencing which may identify and subtype known pathogens as well as novel hitherto unrecognized pathogens. The latter approach will leapfrog pathogen discovery and likely the identification of known and novel pathogens causing outbreaks of unknown etiology.

Main messages

- Future and near term deployable using shot gun metagenomics (with MIDAS/MaxBin reference based/reference free binning) and highly multiplexed amplicon sequencing for subtyping directly from a clinical specimen.
- Metagenomics shotgun sequencing and highly multiplexed amplicon sequencing approaches can be used to better understand unknown diarrheal diseases, including looking at viruses and parasites and whether there are more than one source contributing to illness.

Genomes from Metagenomes
Stephan C. Schuster, Nanyang Technological University, Singapore

Microbial communities constitute the largest domain of life in terms of biomass, however, less than 1% of all microbial organisms are believed to be cultivatable. With the onset of next-generation sequencing surveys of non-cultivatable microbial assemblages have become accessible by metagenomics analysis. This, however, only results in fractions of the total genomic information of a given community and does not allow for the identification of cellular genome information. In this presentation, I will demonstrate several methods that allow dissecting microbial communities by single-cell techniques in combination with single cell sequencing. In addition, the generation of genomes from metagenomes by deep sequencing and assembly of individual genomes from metagenomics data is presented.

Main messages

- References of unknown organisms and plasmids from are currently being generated for inclusion in the database
- Metagenomic deconvolution is complicated but increasingly possible

WGS – the One Health Linkage
Eric Brown US Food & Drug Administration, United States of America

Whole genome sequencing now regularly underpins public health surveillance and food safety traceability efforts in the US and other countries where disease outbreak monitoring and response programs are commonly implemented. One expected and welcome shift in the application of WGS to food and feedborne safety has been a much more detailed understanding of the natural reservoirs and avenues of dissemination of human enteric pathogens throughout the farm to fork continuum, oft times including environmental niches, livestock and other agricultural settings, as well as water and food sources directly linked to human consumption and exposure. Thus, the agnostic nature and widespread availability of WGS data has made genomic sequencing an excellent tool for integration and surveillance in a One Health approach. The GenomeTrakr global foodborne pathogen database further advances this effort by providing a consolidated open-source and curated WGS database for tens of thousands of food and environmental bacterial pathogen sequences. Culture dependent examples of this approach are well established and include WGS-based pathogen links between agricultural waters and the human food supply as well as linkage of drug resistance and other adaptive changes in Salmonella that ensure its survival throughout this continuum. Moreover, culture-independent or "quasi" independent WGS-based metagenomic approaches are starting to provide such One Health linkages directly from food and environmental samples. The emerging role of direct WGS and a novel "metaGenomeTrakr" database in this area will be discussed.

Main messages

- WGS for traceability is now standard operating procedure and the next step is moving beyond to use it for understanding metagenomics and functional genomics.
- Metagenomics will provide solutions to One Health challenges and in the food safety microbiology sector.
- The Metagenome Trackr is a new and open database sharing metagenomic data of entire microbial communities and microbiome currently being piloted.

Main Q&A messages

- Culture independent systems are a likely possibility in the near future.

Virome Profiling of Sewage for Human Disease Surveillance
My Phan, The Erasmus University Medical Center, The Netherlands

Detecting viruses in sewage might be a big game changer for viral surveillance. If the detection methods are sensitive enough, the proponents argue, we could detect viruses in sewage at early times before a large number of people are infected. What is missing from these discussions are some concrete numbers on the quantity of viral sequences that might be found in sewage, the dilution and the degradation of the viruses that that may occur between the patient and the sewage collection point, the stability of disease-causing viruses in sewage and the challenges of assembling useful viral sequence contigs from essentially pooled samples. I will discuss some of our experiences applying next-generating sequencing (NGS) to detect and quantify the viral sequence content of sewage, using the global sewage samples collected from 63 countries.

Main messages

- Agnostic deep viral sequencing of sewage allows the characterization of total viruses from multiple host species e.g. human, animals, plants, insects in large populations.
- As sewage samples are rich in biological content, there is a need to concentrate viral content during processing to eliminate bacteria and obtain positive viral reach.
- While certain challenges exist in accurate classification of complex short reads data and contigs, sewage data can still provide a lot of information for outbreak modelling and prediction when there is longitudinal sampling.

One Health Surveillance and Risk Prediction in Influenza
Ron Fouchier, The Erasmus University Medical Center, The Netherlands

Today, genome sequence data are available from public databases for well over 200,000 influenza virus strains from humans and animals around the world. Increasingly, this dataset is used to inform policy, for instance for epidemiological tracing during surveillance studies. More and more, the data is also used to infer changes in virus traits that are important for public and animal health. Immune escape of human influenza viruses ("antigenic drift") is increasingly monitored primarily by NGS during epidemics, followed by phenotypic confirmation of newly emerging variants to identify new potential vaccine candidates. Likewise, during influenza outbreaks in animals, viral genotypes are used to predict viral phenotypes associated with antiviral resistance or pathogenic, zoonotic or pandemic risks. I will discuss the current state-of-the art with respect to influenza virus genome sequencing and prediction of viral phenotypes from the genomic data during surveillance studies.

Main messages

- Rapid high-throughput functional surrogate assays have become available for several viral phenotypes e.g. NA-STAR, HA receptor specificity, HA temp/Ph stability, HI etc.
- The time and cost of conducting surveillance studies on viruses are being reduced due to replacing some phenotype assays with genome sequencing and more affordable and user friendly technology.
- Sequence data alone can provide inference on several important phenotypes but confirmatory testing remains key.

Bringing NGS to Diagnostic Virology
Sander van Boheeman, The Erasmus University Medical Center, The Netherlands

Next-generation sequencing (NGS) has substantially improved the possibilities in clinical virology. Within one sequencing run NGS can perform a wide variety of diagnostic assays, e.g. detection and discovery, genotyping, resistance mutations, virulence markers, and transmission markers. However, many hurdles need to be overcome to implement NGS in routine testing in the clinical laboritory. Turnaround times of many sequencing platforms are not competitive with the turnaround time needed to inform clinical practice and hospital epidemiology. Large datasets of reads are difficult to analyse and store, often needing specialised expertise to manage. In metagenomic sequencing a significant amount of human sequences are created, leading to privacy issues. Other examples are contamination, database usage and internal control. In this presentation, some of the key challenges and possible solutions will be discussed.

Main messages

- Various approaches exist in bring NGS to diagnostic virology (1) direct metagenomics sequencing (2) PCR amplicon sequencing (3) target enrichment sequencing.
- Defining the purpose of the assay and the available resources are imperative in order to use the right data analysis algorithm e.g. no knowledge of sample required for (1) but high amount of sequencing required to filter out background.

Fast and Cost-effective Sequencing of RNA Virus Genomes in Clinical Samples
Ramette A*, Grädel C, Terrazos Miani MA, Barbani MT, Steinlin-Schopfer J, Bittel P, Suter FM, Leib SL, University of Bern, Switzerland

Human enteroviruses are small RNA viruses that affect millions of people each year and lead to a variety of symptoms ranging from mild illness to severe neurological disorders. Those viruses are routinely diagnosed by PCR assays, often combined with partial sequencing for genotyping. Due to high genomic variability, PCR-based approaches can lead to false negative results. Whole-genome sequencing (WGS) provides complete genetic information, but the approach is more expensive and time consuming, and not routinely used in diagnostic laboratories. Nanopore sequencing offers now the possibility for fast WGS and also enables direct RNA sequencing. We developed a wet lab protocol and bioinformatics pipelines for fast WGS of enteroviruses in clinical samples. WGS of enterovirus RNA genomes were performed from cell cultures and directly from clinical stool samples using a MinION nanopore sequencer, a small portable device allowing long reads and real-time acquisition of sequences. We compared the accuracy of both amplified cDNA and direct RNA sequencing with that of Sanger sequencing, and determined the duration of the nanopore sequencing and the bioinformatic steps needed to obtain the highest consensus accuracy. Nanopore sequencing of cDNA molecules from enteroviruses grown in cell cultures readily provided >95% coverage of enterovirus genomes (e.g. CV-A6, CV-A16, E18), and >99% consensus accuracy as compared to Sanger sequencing. Direct RNA sequencing of total RNA extracted from enterovirus-positive stool sample obtained in June 2017 provided very long reads, often covering the near-complete RNA genome of a Coxsackievirus A6 strain. Comparison of manual extraction (Trizol) vs. automated extraction (Easymag, Biomerieux) indicated that direct RNA sequencing is compatible with automated extraction procedure. Yet, high RNA loading quantities are needed to successfully sequence RNA natively, affecting the sensitivity of the approach. Extra polishing steps using nanopolish software were required to obtain higher consensus accuracies. Enterovirus genomes extracted directly from clinical samples were successfully sequenced using the nanopore sequencing technology. cDNA sequencing provides long, high quality reads and RNA direct sequencing enables the fastest turnaround time for identifying enteroviruses in clinical samples. This study demonstrates the usefulness of fast sequencing technology in the diagnostic laboratories.

Main messages

- Direct RNA sequencing is more suited for application in rapidly identifying RNA viruses from patient samples.
- Sequencing of amplified cDNA is more suited for application in epidemiological analysis.

Viromes As Genetic Reservoir for the Microbial Communities in Food-Associated Environments: A Focus on Antimicrobial- Resistance Genes

Diego Mora Department of Food Environmental and Nutritional Sciences, University of Milan, Italy

The description of viral populations was carried out in three different food-related environments with a focus on the identification and mobilization of antibiotic resistance genes (ARGs). Here reported three different cases of study that involve a comprehensive characterization of viral and microbial communities and the identification of ARGs in virome and microbiomes. In particular, we have characterized: i) water sample from an experimental aquaculture plant, ii) viral communities present in the air surrounding cheese production area in two different dairy plants, and iii) water samples from Lambro River characterized by different levels of urbanization. Shotgun metagenomic sequencing was used to study both microbes and viruses, while 16S rRNA profiling analysis completed the characterization of the microbial community. The three different cases of study showed how antibiotic resistance genes are wide spread in different environments irrespective of the presence of associated anthropic activities. In this context, we hypothesize that the release of antibiotics molecules in the environment by the microbiota is a driving force able to maintain ARGs in the microbiome and the consequent mobilization in the virome. Identification of ARGs and, more in general, all the microbial genes identified in the viromes of the different cases of study did not reflect the microbiome taxonomy, thus suggesting that microbial genes mobilized in the genome of viruses should be considered as a reminiscence of the past recombination events rather than a picture of the current microbial diversity. Beyond that, the analysis of the distribution of the different metagenomic profiles underlined that the variations both in terms of presence of genes and in terms of abundances are more visible in viromes than in microbiome that are less susceptible to gene fluctuation.

Main messages

- The virome was found to be more sensitive in detecting small changes in the environment i.e. ARGs compared to associated microbiomes
- There is potential for the use of metagenomics to identify food safety and spoilage concerns and to assist in the effective implementation of preventative controls

Proficiency Testing for Viral High-throughput Sequencing

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High-throughput sequencing (HTS) has enabled the identification of several viruses and is becoming increasingly important in clinical settings and disease outbreaks. The presented virus proficiency test (PT) was designed to contribute to the standardization of HTS "wet lab" and "dry lab" procedures, with the main goal to improve the detection, identification and analysis of viral pathogens in diagnostic samples. The participants' proficiency in identification and analysis of viral pathogens in complex samples and HTS datasets was evaluated in two general parts of the virus PT: (1) the laboratories' proficiency to analyze HTS data and identify viral pathogens, (2a) the laboratories' sample preparation and sequencing procedures and (2b) the laboratories' HTS performance and output. Therefore, participants of the PT were invited to (1) analyze a complex metagenomics fastq dataset to identify viral sequences of 4 viruses in varying numbers, (2a) perform purification, DNA/RNA extraction, library preparation and sequencing of a virus strain within a complex sample matrix and (2b) perform library preparation and sequencing of viral RNA/DNA. The results summarized in this talk indicate that numerous protocols, tools and different workflows are used for virus HTS and results of such workflows differ in sensitivity and specificity. So far, there are no standard procedures for sample preparation and virome analyses, and sharing and comparing reliable results of such analyses remain difficult.

Main messages

Dry lab

- 14 participants involved
- Analysis times varied significantly
- No correlation between computer/server, sensitivity, database
- Detection of mutated viruses highly challenging
- New dry lab prepared in collaboration with European Virus Bioinformatics Center is offered to GMI

Wet lab

- 8 participants involved
- Good identification rates
- Organizer to provide clearer instructions
- New wet lab scenario oriented on skin diseases offered to GMI

Main Q&A messages

- Bacteriophages were detected in less abundance from sewage than in human stool samples and the reason for this is yet to be confirmed.

GMI Working Group Outcomes

Work Group 1: Political challenges, outreach and building a global network

A strategic plan leading up to the World Health Assembly 2020 will be drafted, with the ultimate aim of including a WGS data-sharing resolution on the agenda. The plan will include a further development of a potential draft resolution text at GMI 12. For any related regional conferences attended by WHO and FAO, they will endeavor to mention GMI's work and explain why the WHA 2020 resolution text is being suggested. Following on from the first GMI letter, a second letter will be drafted and sent to countries that responded as well as other interested countries. The letter will state a request for countries to support the drafting of WHA 2020 resolution text. For ease of communication the letters will be translated into Spanish and French by WG1 members that have volunteered. In the upcoming WHA 2019, there will be a discussion on AMR. It is planned to suggest inclusionof WGS data sharing in the resolution text on AMR through directly corresponding with UK and China contacts responsible for drafting the text.
Funding considerations for the development of the GMI and centralized data storage, must be discussed, e.g. considering funding models of UN, CERN, private, crowdfunding. While the US has conducted its own cost benefit analysis, the analysis should be conducted to generate results from the global perspective. A SWOT analysis should also be created to address the fear faced by several entities, of sharing data, specifically WGS data. Funding to support the travel of developing country participants to GMI 12 must also be looked into.

It should be noted the urgency to act on GMI's goals is also relevant to developing countries. If all countries, NGOs and IGOs i.e. WHO, FAO are not wholly engaged in some way in the near future, the current status quo will continue and many countries will not have input into what is ultimately adopted.

Work Group 2: Repository and storage of sequence and meta-data

During GMI7-8 it was agreed to publish a paper describing the minimum data for matching which includes the sequence data and metadata, and describing harmonization of the metadata template. A draft has now been put together and the action item will soon be checked off. Furthermore, the group wanted to notethat there is a way to flag an isolate that is a part of an outbreak retrospectively, which will be useful for those developing various bioinformatic tools and bioassays. It was also discussed that in the event there are strains being sent to other groups who then resequence them and lose their registration to the original strain, there needs to be a "best practice" pointing back to the original strain in the metadata so it is acknowledged that these are not completely independent strains but originate from a reisolation /reculture.

Data exchange for antibiograms was also discussed - in some veterinary cases the current metadata template doesn't capture host and tissue specificity e.g. may occur in some animals that have different cutoffs for antibiotics depending on which tissue it was obtained from. It was agreed that modifications need to be made for the aforementioned case.

Epidemiologic cutoff values (measures of a drug MIC distribution that separate bacterial populations into those representative of a wild type population, and those with acquired or mutational resistance to the drug) were discussed and it was agreed that modifications may need to be made on the

antibiotic template for the clinical, non clinical isolates for the wild type versus non wild type, possibly expanding that list to include those options.

Work Group 3: Analytical approaches

Work group 3 consists of five subwork groups.

The steering subgroup decided to communicate the successes of work group 3 members by dedicating a channel on the GMI website. Work group 3 members who have published relevant manuscripts can share this on the channel by sending it to Ruth Timme. Subgroup 2 have decided to add five new benchmark datasets that may be useful for the community e.g. dataset for different platforms (not only MiSeq). Subgroup 3 is focused on benchmarking pipelines and metrics. They have decided to come up with metrics for cluster detection comparison and to provide these for the community e.g. specific formula for specific purpose to calculate average coverage, no. of contigs etc. They are also interested in having community benchmarking for cluster detection pipeline tools and in GMI13, to add the community benchmarking for variant calling. Subgroup 4 is looking to tackle the analysis of metadata for integrating metadata analysis into some of the analysis pipelines, the goal of which is to be creating templates. Subgroup 5 is focused on analytical approaches specific to metagenomics. Their goal is to address data sharing and privacy issues for the metagenomics datasets especially around human sequencings in them, environmental scan for wet and dry lab approaches for different applications.

Work Group 4: Ring trials and quality assurance

Ring trial for bacteria – after 2 ring trials, the group will take a break to analyse and publish data and come up with next proficiency test for 2019. This will include running a survey among participants to address improvements for the next proficiency test. It was highlighted that DTU is not equipped to independently analyze all of the data and any parties who wish to contribute should email Frank Aarestrup, fmaa@food.dtu.dk.

Ring trial for virus – in late summer/early autumn a new dry lab PT will be shared with GMI. A wet lab PT will follow. A survey may also be conducted in advance to navigate the desire of the participants.

Concluding Discussion

The strengthened linkages with the World Health Organization through the coordinated planning of the 11th GMI meeting has been a progressive step for GMI. Following from this, and building on the similar support from FAO at GMI9, the GMI Steering Committee will consider writing a review paper on GMI and its history. The following suggestions and remarks were made in relation to future meetings:

- There should be more time for discussion. Work groups should meet more than once, ideally on Day 1 and on Day 3.
- Work groups should be more involved in programme development
- GMI 12 meeting could be tied to training/outreach e.g. such as in the case of the 2016 INFOSAN meeting which was tied to a WGS workshop, maybe only bioinformatics hands on training,
- A networking session outside of the meeting e.g. dinner reception would also be useful to build up contacts
- GMI 12 should focus on metagenomics. The presentations could also focus more on the power and persuasion of the technology, rather than discussing only the finite technical details it could go beyond to tell the story of the adopters. Low-middle income countries could also be invited to present their success stories. A session on a political level inviting non-technical people on board could also be considered. A presentation on the history and accomplishments of GMI and the plans will help to hold work groups accountable for their goals as well as better measure progress e.g. what is the setup, who are the drivers, specifically who is the steering committee, description of the charter and a revisit of this all. The mission and vision need to be discussed to ensure it is in sync with how sequencing has evolved over the past seven years.